JHeimbach LLC

February 11, 2014

Paulette Gaynor, Ph.D.
Senior Regulatory Project Manager
Division of Biotechnology and GRAS Notice Review (HFS-255)
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740

Dear Dr. Gaynor:

Pursuant to proposed 21 CFR 170.36 (62 FR 18960; April 17, 1997), DSP GOKYO FOOD & CHEMICAL Co., Ltd., through me as its agent, hereby provides notice of a claim that the use of tamarind seed polysaccharide described in the enclosed notification document is exempt from the premarket approval requirement of the Federal Food, Drug, and Cosmetic Act because DSP GOKYO has determined that the intended use is generally recognized as safe (GRAS) based on scientific procedures.

As required, three copies of the notification are provided. Each copy also includes the signed conclusion of the members of the GRAS expert panel.

If you have any questions regarding this notification, please feel free to contact me at 804-742-5548 or jh@jheimbach.com.

Sincerely,

(b) (6)

James T. Heimbach, Ph.D., F.A.C.N.
President

Encl.

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Determination of the GRAS Status of the Addition of Tamarind Seed Polysaccharide to Conventional Foods as a Stabilizer and Thickener

Prepared for
DSP GOKYO FOOD & CHEMICAL Co., Ltd.
Osaka Japan

Prepared by
JHEIMBACH LLC
Port Royal VA

February
2014

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1. GRAS EXEMPTION CLAIM

DSP GOKYO FOOD & CHEMICAL Co., Ltd., through its agent JHEIMBACH LLC, hereby notifies the Food and Drug Administration that the use of tamarind seed polysaccharide in conventional foods as described below is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because DSP GOKYO has determined through scientific procedures that this use is generally recognized as safe (GRAS).

(b) (6)

2/11/14

James T. Heimbach, Ph.D., F.A.C.N.
President, JHEIMBACH LLC

Date

1.1. Name and Address of Notifier

Contact: Yuiko Homma
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1.2. Name of GRAS Substance

The common name of the substance that is the subject of this GRAS notice is tamarind seed polysaccharide (also referred to as tamarind seed gum) sold under the brand names GLYLOID and GLYATE. This substance, obtained from *Tamarindus indica* L., is a xyloglucan, composed of a linear chain of β(1-4)-linked glucose residues, to about 75% of which are attached α(1-6)-linked xylose units, some of which are further linked to galactose residues by β(1-2) bonds.

1.3. Intended Use and Consumer Exposure

Tamarind seed polysaccharide is intended to be used as a thickener, stabilizer, emulsifier, and gelling agent in 12 food categories including ice cream, sauces and condiments, dressings and mayonnaise, fruit preserves, desserts, beverages, pickles, tsukudani, spreads and fillings, flour products, soup, and all other food categories, with addition levels ranging from 0.2 to 1.5%. Based on food consumption data from the National Health and Nutrition Examination Survey (NHANES), the estimated mean and 90th percentile daily intakes of tamarind seed polysaccharide are 2.57 and 4.43 g, respectively. Expressed in terms of bodyweight, the estimated mean and 90th percentile daily intakes are 44.9 and 91.0 mg/kg bw, respectively.

1.4. Basis for GRAS Determination

DSP GOKYO's GRAS determination for the intended use of tamarind seed polysaccharide is based on scientific procedures as described under 21 CFR §170.30(b).

Determination of the safety and GRAS status of the addition of tamarind seed polysaccharide to conventional foods was made through the deliberations of an Expert Panel consisting of Joseph F. Borzelleca, Ph.D., Walter H. Glinsmann, M.D., and Robert J. Nicolosi, Ph.D., who reviewed the information in this monograph as well as other information available to them. These individuals are qualified by scientific training and experience to evaluate the safety of food ingredients. They critically reviewed and evaluated the publicly available information, including the potential intake of tamarind seed polysaccharide, and individually and collectively concluded that the available information on tamarind seed polysaccharide contains no evidence that demonstrates or suggests reasonable grounds to suspect a hazard to the public health under its intended conditions of use.

It is the Expert Panel's opinion that other qualified scientists reviewing the same publicly available data would reach the same conclusion. Therefore, the addition of tamarind seed polysaccharide to conventional foods under the conditions of use described is GRAS by scientific procedures.

1.5. Availability of Information

The data and information that serve as the basis for the GRAS determination will be sent to the FDA upon request, or are available for the FDA's review and copying at reasonable times at the office of James T. Heimbach, Ph.D., President, JHEIMBACH LLC, 923 Water Street, P.O. Box 66, Port Royal VA 22535, telephone 804-742-5548 and e-mail jh@jheimbach.com.

1.6. Abbreviations Used in This Document

CAS = Chemical Abstracts Service
cGMP = current good manufacturing practice
DHHA = U.S. Department of Health and Human Services
EDI = estimated daily intake
FDA = Food and Drug Administration
GRAS = generally recognized as safe
i.p. = intraperitoneal
mPa·s = millipascal-second
N = newton
NHANES = National Health and Nutrition Examination Survey
NOAEL = no observed adverse effect level
OECD = Office of Economic Cooperation and Development
USDA = U.S. Department of Agriculture

2. IDENTITY OF THE SUBSTANCE

2.1. Chemical Name

The substance that is the subject of this GRAS notification is tamarind seed polysaccharide, also referred to as tamarind seed gum, derived from *Tamarindus indica* L. This substance is a xyloglucan, composed of a linear chain of β(1-4)-linked D-glucan residues that is partially substituted with α(1-6)-linked D-xylopyranose, some of which are β-D-galactosylated at O-2. Savur and Sreenivasan (1948) calculated the proportions by weight of the three sugar residues as 55.4% glucose, 28.4% xylose, and 16.2% galactose, corresponding to a molar ratio of 3:2:1 glucose:xylose:galactose.

2.2. Trade or Common Names

The trade names of tamarind seed polysaccharide are GLYLOID and GLYATE.

2.3. CAS Registry Numbers

Tamarind seed polysaccharide has Chemical Abstracts Service (CAS) registry number 39386-78-2.

2.4. Structural Formulas

A generalized structure is shown in Figure 1.

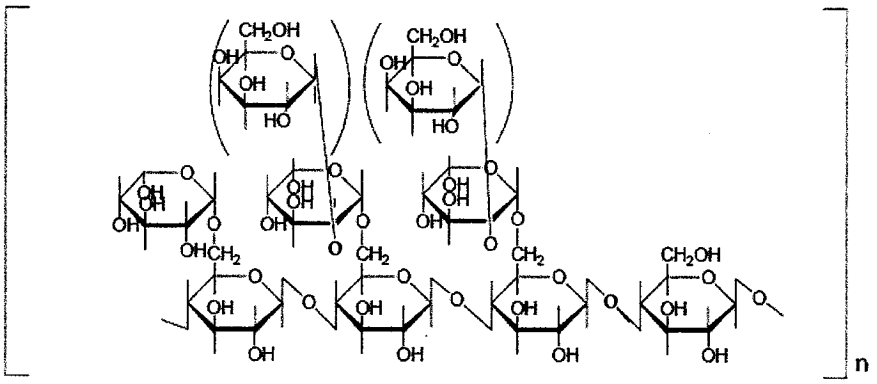

Figure 1. Generalized Structural Formula of Tamarind Seed Polysaccharide.

Tamarind seed polysaccharide consists mainly of four types of oligosaccharides as repeating units (Sone and Sato 1994; Nishinari et al. 2009). These units include a heptasaccharide (Glu_4Xyl_3), two varieties of octasaccharide (Glu_4Xyl_3Gal), and a nonasaccharide ($Glu_4Xyl_3Gal_2$), found in a ratio of 13:39:48. These structures (Urakawa et al. 2002) are shown in Figure 2. Based on the results of light scattering and small-angle X-ray scattering, Urakawa et al. (2002) determined that tamarind seed polysaccharide is essentially linear.

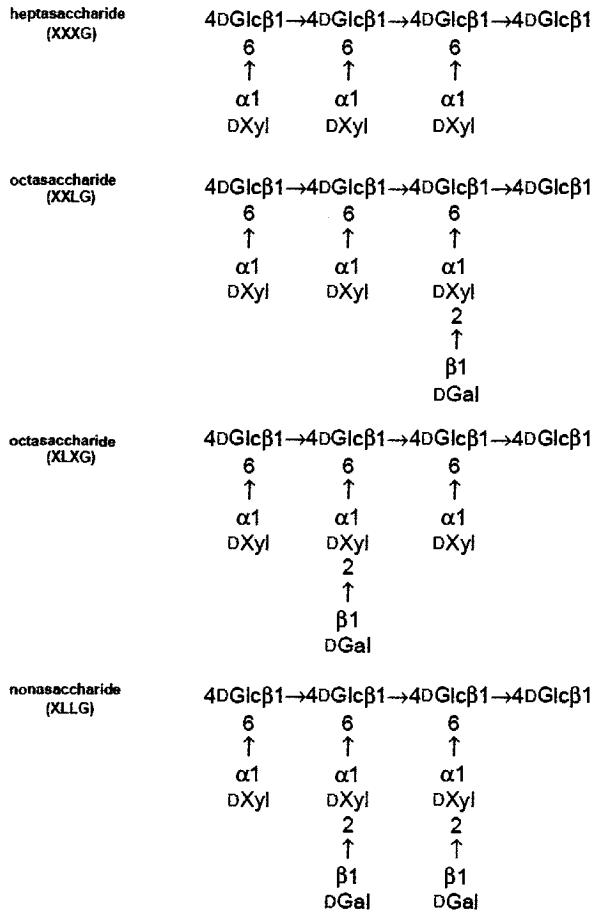

Figure 2. Structural Formulas of Four Varieties of Repeating Units in Tamarind Seed Polysaccharide (from Urakawa et al. 2002).

2.5. Physical and Chemical Properties

The reported range of molecular weights for tamarind seed polysaccharide is large: 115 kDa (Glicksman 1986), 470 kDa (Kato 2000), 650 kDa (Nishinari et al. 2000), 880 kDa (Gidley et al. 1991), 1160 kDa (Dentini et al. 2001), and 2500 kDa (Lang and Kajiwara 1993). Several explanations have been offered for this extreme range, including variations in sample preparation procedures (Lang et al. 1993) and the tendency of xyloglucans to self-associate, thus exaggerating weight-averaged estimates of molecular weight (Nishinari et al. 2009).

The viscosity characteristics of tamarind seed polysaccharide have been extensively studied. The following figures illustrate the viscosity behavior of GLYLOID 2A, a brand name product produced by DSP GOKYO FOOD & CHEMICAL Co., Ltd.

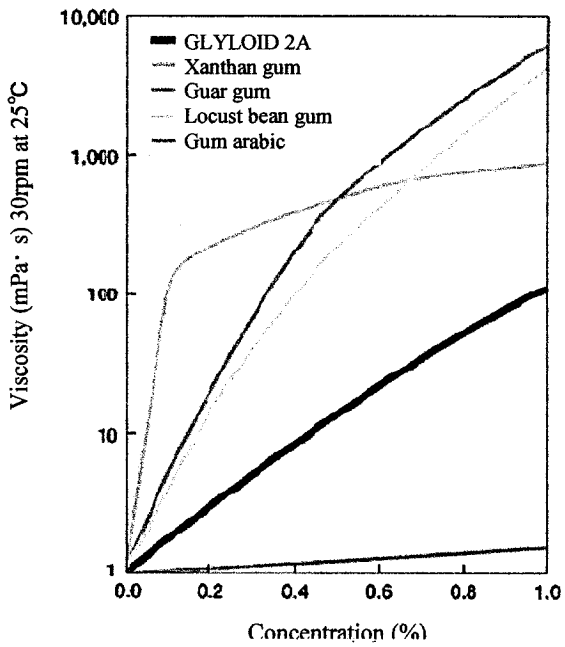

Figure 3. Viscosity and Concentration.

As can be seen in Figure 3, tamarind seed polysaccharide exhibits moderate viscosity with a linear dependence on concentration. On the other hand, its viscosity is negatively correlated with temperature and is independent of the intensity of shear or stirring force (Figures 4 and 5).

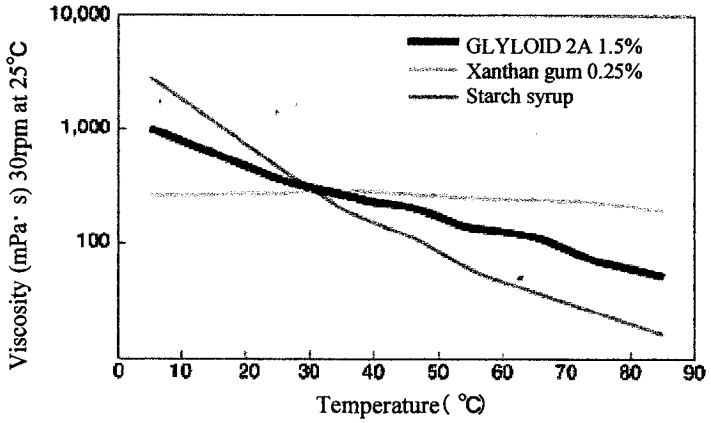

Figure 4. Viscosity and Temperature.

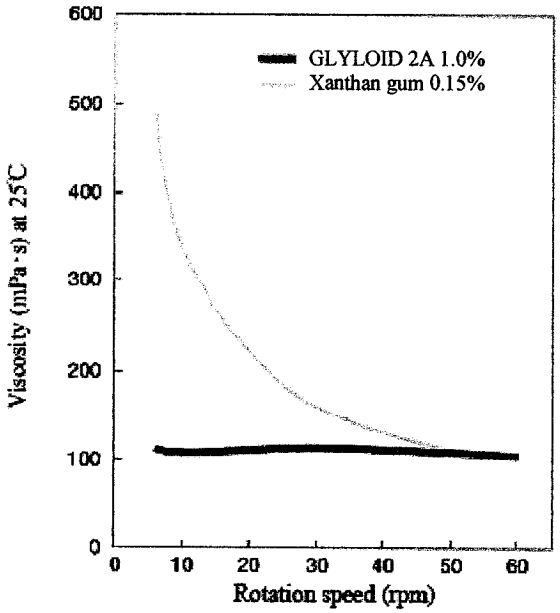

Figure 5. Viscosity and Shear Force.

Tamarind seed polysaccharide shows stable viscosity over a wide range of pH, during heating of up to 100°C for up to 2 hours, and during storage even at low pH (Figures 6-8).

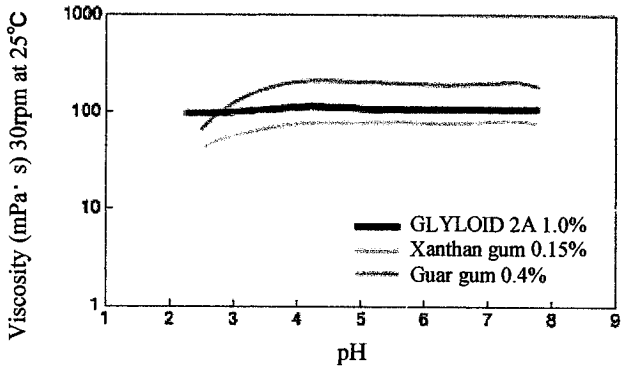

Figure 6. Viscosity and pH.

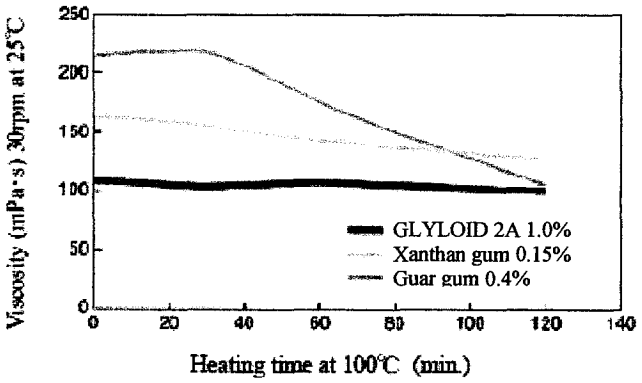

Figure 7. Viscosity and Heating.

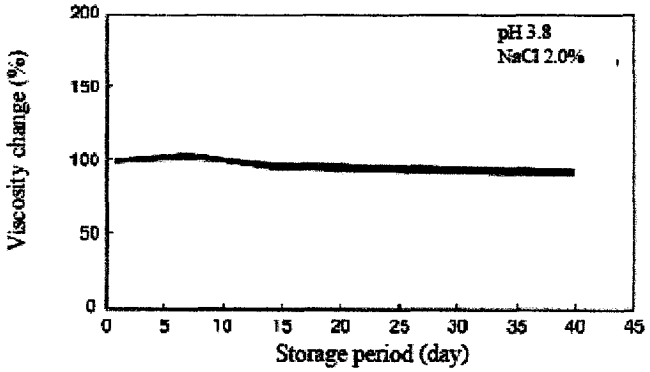

Figure 8. Viscosity during Storage.

2.6. Botanical Source

According to the *Handbook of Legumes of World Economic Importance* (Duke 1981), tamarind (*Tamarindus indica* L.) is a long-lived large evergreen tree that grows to a height of 33 m with a trunk thickness of 1.5-2 m and is widely distributed in the subtropical and tropical zone (Duke 1981; Morton 1987; El-Siddig et al. 2006). The bark is brownish-gray and the leaves are 5-12 cm in length. The fruit (which is processed into soft drinks, jams, desserts, curries, and other foods and widely consumed in Asia) appears as a brown pod 5-18 cm long and 2.5 cm wide containing 1-12 seeds (Figure 9). The seeds are smooth and glossy flattened oblongs that contain about 65-72% polysaccharide along with about 15-23% protein and 4-7% lipids, but no free sugars. *Tamarindus* is a monotypic genus, containing the sole species *T. indicus*.

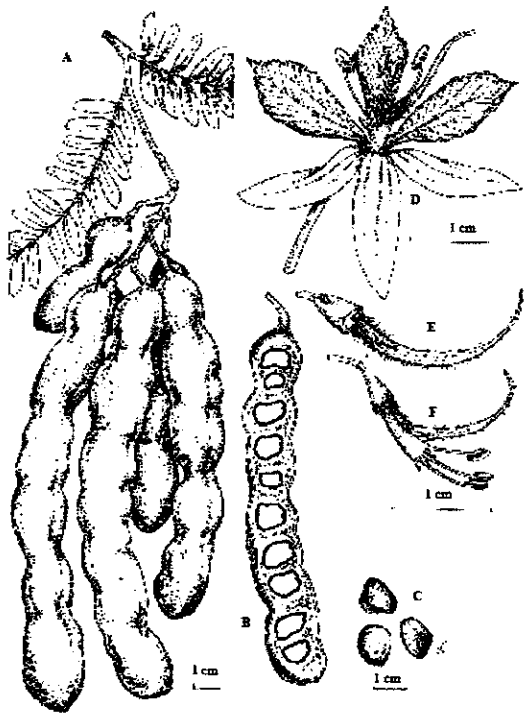

Figure 9. Flowers, Pods, and Seeds of Tamarind (El-Siddig et al. 2006).

2.7. Production Process

2.7.1. Starting Material

Seeds of the sour tamarind are obtained from the northern, northeastern, and central areas of Thailand and from southern and central India. No pesticides are applied during cultivation of these trees, which have strong natural resistance to infestation.

The composition of three samples of ground decorticated tamarind seed[1] (meal) was measured by Savur and Sreenivasan (1948), with the findings shown in Table 1 (with an added column showing the means).

Table 1. Composition of Tamarind Seed (from Savur and Sreenivasan 1948).

Component	Sample 1	Sample 2	Sample 3	Mean
Polysaccharide (%)	58.47	62.88	63.30	61.55
Crude fiber (%)	4.93	1.46	2.60	3.00
Sugars (%)	--	--	--	--
Crude protein (%)	15.69	15.28	15.35	15.44
Ash[1] (%)	8.09	10.55	9.00	9.21
Moisture (%)	12.64	10.80	11.30	11.58
1. Present as Na, K, Ca, Mg, P, and Si.				

[1] The testa or coat constitutes about 30% of the whole seed, and so the decorticated seed meal is about 70% of the total seed.

2.7.2. Processing Method

2.7.2.1. Phase 1: Extraction of Tamarind Kernel Powder

The black tamarind seeds are sieved and roasted. After cooling, the roasted seeds are placed in a rotary mixer, which removes the black testa (20-30% of the seed) to leave a light brown to white endosperm or kernel. This kernel is visually sorted and any off-color kernels are removed, after which the kernels are polished in a rotary mixer and cut in a finger cutter. The cut kernels pass through a hammer mill and are sifted with a 200-mesh filter. The product of these processing steps is tamarind kernel powder consisting primarily of polysaccharide with residual protein, lipid, and minerals. It contains no more than 10.0% moisture and has a viscosity of not less than 450 millipascal-seconds (mPa·s).

2.7.2.2. Phase 2: Separating and Refining of Polysaccharide

Tamarind kernel powder is stirred into a solution of food-grade methyl alcohol. After stirring, food-grade sodium hydroxide is added and the mixture is again stirred at a controlled temperature. The polysaccharide is separated from the protein, lipid, and minerals by centrifugation and food-grade citric acid is added as needed to adjust the pH to the desired level. The polysaccharide is dried, pulverized, and sieved through a screen. This process is used to produce a polysaccharide sold under the brand name GLYLOID 2A.

A product that is more soluble in cold-water, sold under the brand name GLYLOID 3S, is produced by heating the polysaccharide and rinsing it in a methyl alcohol solution to remove the colored material prior to pH adjustment with citric acid.

A third product, partially acid-hydrolyzed to produce a lower viscosity polysaccharide, is sold under the brand name GLYATE. This product is treated with food-grade sulfuric acid until hydrolysis has resulted in the desired viscosity, then neutralized with food-grade sodium hydroxide, sieved, and rinsed in a methyl alcohol solution. It is then dried, pulverized, and sieved in the same manner as the two GLYLOID products.

2.8. Food-Grade Specifications for Tamarind Seed Polysaccharide

DSP GOKYO FOOD & CHEMICAL Co. has established food-grade specifications for their tamarind seed polysaccharide products. The specifications for GLYLOID 2A, GLYLOID 3S, and GLYATE are nearly identical, differing primarily in the viscosity; GLYLOID 2A has a range of 400 - 600 millipascal-seconds (mPa·s), GLYLOID 3S has a range of 500 - 800 mPa·s, and GLYATE has a range of 10 – 100 mPa·s. Five lots of each product were analyzed to demonstrate consistent compliance with the specifications with the results shown in Tables 2, 3, and 4.

The specifications set for GLYLOID 2A and 3S and for GLYATE do not include limits for mercury and cadmium; nevertheless the levels of these heavy metals were assessed in each of the 5 test lots of each product and both were consistently below the detection level of 0.01 mg/kg. Methanol residues are also tested regularly by headspace gas chromatography and are consistently found to be under 50 mg/kg.

Table 2. Food-Grade Specifications for GLYLOID 2A.

Parameter	Specification	Lot No.				
		12.12.01-2	12.12.02-3	12.12.03-1	12.12.05-5	12.12.06-3
Physical form	Powder	Pass	Pass	Pass	Pass	Pass
Color	White to light brown	Pass	Pass	Pass	Pass	Pass
Odor	None to slight	Pass	Pass	Pass	Pass	Pass
Viscosity (mPa·s[1])	400 – 600	516	494	528	550	514
Gel strength (N[2])	NLT[3] 2.0	4.1	4.1	4.2	4.3	4.3
Loss on drying (%)	NMT[4] 7.0	2.3	2.3	2.4	2.1	2.1
Ash (%)	NMT 5.0	0.4	0.4	0.4	0.4	0.5
Protein (%)	NMT 3.0	1.3	1.3	1.3	1.2	1.2
Lipid (%)	NMT 1.0	0.3	0.3	0.3	0.3	0.3
Lead (mg/kg)	NMT 2	<2	<2	<2	<2	<2
Arsenic (mg/kg)	NMT 1	<1	<1	<1	<1	<1
Viable aerobes (cfu[5]/g)	NMT 2000	<100	<100	<100	<100	<100
Heat-resistant aerobes (cfu/g)	NMT 1000	<100	<100	<100	<100	<100
Coliforms (in 0.1 g)	Negative	Negative	Negative	Negative	Negative	Negative

1. millipascal-seconds
2. newtons
3. not less than
4. not more than
5. colony-forming units

Table 3. Food-Grade Specifications for GLYLOID 3S.

Parameter	Specification	Lot No.				
		12.11.06-2	12.11.07-2	13.01.10-1	13.01.13-2	13.01.16-1
Physical form	Powder	Pass	Pass	Pass	Pass	Pass
Color	White to light brown	Pass	Pass	Pass	Pass	Pass
Odor	None to slight	Pass	Pass	Pass	Pass	Pass
Viscosity (mPa·s[1])	500 – 800	637	633	629	683	649
Gel strength (N[2])	NLT[3] 2.0	4.2	4.1	4.2	4.6	4.2
Loss on drying (%)	NMT[4] 7.0	0.9	0.8	0.9	0.9	0.9
Ash (%)	NMT 5.0	0.5	0.5	0.5	0.5	0.5
Protein (%)	NMT 3.0	0.3	0.3	0.4	0.2	0.3
Lipid (%)	NMT 1.0	0.1	0.1	0.1	0.1	0.1
Lead (mg/kg)	NMT 2	<2	<2	<2	<2	<2
Arsenic (mg/kg)	NMT 1	<1	<1	<1	<1	<1
Viable aerobes (cfu[5]/g)	NMT 2000	<100	<100	<100	<100	<100
Heat-resistant aerobes (cfu/g)	NMT 1000	<100	<100	<100	<100	<100
Coliforms (in 0.1 g)	Negative	Negative	Negative	Negative	Negative	Negative

1. millipascal-seconds
2. newtons
3. not less than
4. not more than
5. colony-forming units

Table 4. Food-Grade Specifications for GLYATE.

Parameter	Specification	Lot No.				
		12.08.18-3	12.08.19-1	13.04.03-1	13.04.03-2	13.04.03-3
Physical form	Powder	Pass	Pass	Pass	Pass	Pass
Color	White to grayish	Pass	Pass	Pass	Pass	Pass
Odor	None to slight	Pass	Pass	Pass	Pass	Pass
Viscosity (mPa·s[1])	10 – 100	29	29	32	35	28
Loss on drying (%)	NMT[2] 7.0	1.3	1.4	1.0	0.7	1.0
Lead (mg/kg)	NMT 2	<2	<2	<2	<2	<2
Arsenic (mg/kg)	NMT 1	<1	<1	<1	<1	<1
Viable aerobes (cfu[3]/g)	NMT 2000	<100	<100	<100	<100	<100
Heat-resistant aerobes (cfu/g)	NMT 1000	<100	<100	<100	<100	<100
Coliforms (in 0.1 g)	Negative	Negative	Negative	Negative	Negative	Negative

1. millipascal-seconds
2. not more than
3. colony-forming units

3. INTENDED TECHNICAL EFFECT

The intended effect of the addition of tamarind seed polysaccharide to food is as a stabilizer and thickener as defined in 21 CFR §170.3(o)(28).

4. INTENDED USE AND CONSUMER EXPOSURE

4.1. Intended Use of Tamarind Seed Polysaccharide

Tamarind seed polysaccharide is intended for use in ice cream, sauces and condiments, dressings and mayonnaise, fruit preserves, desserts, beverages, pickles, tsukudani, spreads and fillings, flour products, soup, and all other food categories. The proposed use levels and technical functions of tamarind seed polysaccharide in these foods are summarized in Table 5.

Table 5. Intended Food Uses of Tamarind Seed Polysaccharide

Category	Examples	Description	Function	Use Level (%)
Ice cream	Ice cream, sorbet, gelato, frozen yogurt	Includes ice cream (regular, light, and fat free types), ice cream novelties/ sandwiches, frozen yogurt, sorbet, fruit juice bars, ices.	Thickener, stabilizer	0.3
Sauces and condiments	Barbecue, steak, demiglace, tomato, chile, tabasco, curry, teriyaki, tare sauces; ketchup; gravy	Includes all types of sauces: barbecue, gravies, tomato-based sauces, teriyaki, ketchup, chili, curry, Worcestershire, tartar, miso, hoisin, soy sauce, mustard, cocktail, etc. and foods containing the same. Excludes sauces from frozen meals.	Thickener, stabilizer	1.0
	Tonkatsu, korokke, yakisoba, okonomiyaki sauces		Thickener, stabilizer	1.5
Mayonnaise and dressings	Mayonnaise, reduced fat mayonnaise; Caesar, French, Italian, ranch, thousand Island, wafu dressings	Includes all mayonnaise and types of salad dressings (regular and low calorie): Caesar, French, Russian, Italian, ranch, thousand island, blue cheese, peppercorn, honey mustard, vinaigrette, and sandwich spreads and dishes containing the same.	Thickener, stabilizer, emulsifier	1.0
Fruit preserves	Fruit spread, jam, jelly, apple sauce	Includes all types of marmalade, jams, preserves, jelly, applesauce, fruit butter and paste, bean paste, and dishes containing the same. Excludes baby food.	Gelling agent, stabilizer	1.0
Desserts	Pudding, Bavarian cream, mousse	Includes all types of ready-to-eat pudding, custard, mousse. Excludes baby food.	Stabilizer	0.2
Beverages	Fruit juice, reduced-fat milk, cocoa drink	Includes low and reduced fat milk, cocoa drinks, fruit juice and fruit drinks (nectar, ades, punches, cocktails, vegetable juices), energy drinks, and sport beverages. Excludes dry, powder mixes, non-reconstituted, frozen forms; fat-free and skim type milk.	Stabilizer	0.2
Pickles	Tsukemono (pickled foods), kimuchi, pickled cucumber, pickled olives, pickled sauerkraut	Includes pickled fruit (apple, mango, olive) and vegetables (tomato, string beans, beets, cabbage, cucumber, okra, mixed vegetables, peppers, radish).	Thickener	1.0
Tsukudani	Laver, mushroom, and kelp tsukudani,	Includes seaweed prepared with soy sauce	Thickener	1.0

Category	Examples	Description	Function	Use Level (%)
Spreads and fillings	Custard cream, spreads	Includes cream/custard cream filling portion of baked goods, all types of pastry filling, cheese spreads, cream cheese, and margarine -like spreads.	Thickener, stabilizer	0.5
Flour products	Bread, pastry, cake, instant noodles, ramen, udon, dough, batter	Includes finished baked goods such as bread, cakes, cookies, brownies, pastries, pancakes, waffles and finished pastas such as macaroni, noodles, spaghetti, lasagna, ravioli, shells, and tortellini. Excludes rice noodles and portion from frozen meals.	Thickener, stabilizer	0.5
Soups	Broth, consume, creamy soups	Broth, consume, and cream soups like tomato, including canned, and condensed, but not dry soup mixes Includes all commercially prepared soups; excludes soups made from home recipes.	Stabilizer	0.2
All other food categories		Includes processed cheese, cheese sauces and cheese sauces from main pasta dishes, imitation cheese, yogurt, ready-to-drink supplement/meal replacement drinks, cream (half and half), whipped cream and non-dairy whipped topping, sour cream, cheese (processed only), cottage cheese, icing and frostings, syrups (chocolate, pancake) and dessert toppings, crackers, all types of candy (chocolates, chocolate covered nuts, chewing gum, candy bars, fruit leather, fondant, marshmallow, fudge), gelatins/jello, dips (guacamole, salsa). Excludes pop-corn, cereal (hot and cold), salty snacks, rice, grain products such as rice, oatmeal, cornmeal, etc.	Thickener, stabilizer, emulsifier, gelling agent	0.5

4.2. Estimated Daily Intake of Tamarind Seed Polysaccharide

Estimated daily intakes (EDI) of tamarind seed polysaccharide were based on food consumption records collected as part of the National Health and Examination Survey conducted in 2003-2004, 2005-2006 (NHANES 2003- 2006). This continuous survey is a complex multistage probability sample designed to be representative of the civilian U.S. population (NCHS 2007, 2008). The NHANES datasets provide nationally representative nutrition and health data and prevalence estimates for nutrition and health status measures in the United States. The NHANES survey over-samples minorities, low income groups, adolescents ages 12-19 years, and adults 60 years of age and older. Statistical weights are provided by the National Center for Health Statistics (NCHS) for the surveys to adjust for the differential probabilities of selection. As part of the examination, trained dietary interviewers collect detailed information on all foods and beverages consumed by respondents in the previous 24 hour time period (midnight to midnight). A second dietary recall is administered by telephone 3 to 10 days after the first

dietary interview, but not on the same day of the week as the first interview. The dietary component of the survey is conducted as a partnership between the U.S. Department of Agriculture (USDA) and the U.S. Department of Health and Human Services (DHHS). DHHS is responsible for the sample design and data collection, and USDA is responsible for the survey's dietary data collection methodology, maintenance of the databases used to code and process the data, and data review and processing. A total of 16,783 individuals in the survey period 2003-2006 provided 2 complete days of dietary recalls. These data were used to estimate two-day average intakes of tamarind seed polysaccharide by individuals aged 2 and older who consumed any of the foods in which the substance is intended to be used.

Estimated daily intakes of tamarind seed polysaccharide per person and per kg bodyweight are shown in Table 6.

Table 6. Estimated Daily Intake of Tamarind Seed Polysaccharide.

Category	Intake per Person (mg/day)		Intake per kg bodyweight (mg/kg bw/day)	
	Mean	90th Percentile	Mean	90th Percentile
Ice cream	239	470	4.4	9.3
Sauces and condiments	605	1389	9.9	23.0
Mayonnaise and dressings	197	443	2.9	6.4
Fruit preserves	262	635	5.6	13.9
Desserts	159	320	2.9	6.1
Beverages	821	1684	16.3	38.5
Pickles	201	422	3.1	6.1
Tsukudani	165	*	2.4	*
Spreads and fillings	147	367	2.2	5.4
Flour products	844	1538	14.1	28.3
Soups	312	602	5.0	10.1
All other food categories	318	735	5.7	13.4
TOTAL	**2570**	**4427**	**44.9**	**91.0**
* Number of consumers insufficient to calculate a 90th percentile of intake.				

Nearly all individuals over the age of 2 years consume foods from at least one of the food categories in which tamarind seed polysaccharide is intended for use; the flour products category alone is consumed by 99.1% of individuals. As can be seen in Table 6, most of the estimated daily intake of tamarind seed polysaccharide is provided by its use in three categories—beverages, flour products, and sauces and condiments. Summing across all intended uses, the estimated mean and 90th percentile daily intakes of tamarind seed polysaccharide are 2.57 and 4.43 g, respectively. Expressed in terms of bodyweight, the estimated mean and 90th percentile daily intakes are 44.9 and 91.0 mg/kg bw, respectively.

5. SAFETY INFORMATION

5.1. Fermentation by Intestinal Bacteria

Tamarind seed polysaccharide, like other xyloglucans, is not digested by human digestive enzymes and may be regarded as part of the dietary fiber fraction of the diet (Yamatoya et al. 2000). It is, however, fermented by the intestinal microbiota (Hartemink et al. 1996). In a series of *in vitro* experiments, Hartemink et al. (1996) found that only 9 strains of bacteria—one bifidobacterium, one bacteroides, and seven clostridia, all commensal strains—were able to degrade the intact polysaccharide. However, after degradation of the polymeric backbone, numerous strains representing a variety of species were able to ferment the resulting oligosaccharides. The authors offered the following model for xyloglucan degradation:

> "Xyloglucan is degraded by endo-β-glucanases, mainly produced by *Clostridium* species, to oligosaccharides. These oligosaccharides are further degraded both by some of the same species, as well as some other bacteria. The complete mechanism of this latter degradation remains to be elucidated. Contrary to many other plant cell wall polysaccharides, xyloglucan is degraded mainly by clostridia, not by bacteroides or bifidobacteria. This may result in a relatively large production of gas after ingestion of this polysaccharide."

It is important to recognize that the clostridia species involved in the degradation of xyloglucans do not include *C. difficile* or other pathogens, but are specialized cellulolytic species such as *C. cellulovorans, C. cellulosi, C. cellulolyticum, C. herbivorans, C. thermocellum, C. cellulofermentans*, and similar species that encode for the appropriate cellulases by means of a cellulosome, an "intricate protein complex consisting of over 20 different catalytic subunits" (Schwarz 2013, Baumann 2007).

5.2. Toxicity

5.2.1. Acute Oral Toxicity

In studies compliant with OECD guidelines for studies of acute oral toxicity, tamarind seed polysaccharide was evaluated in both ddY mice and Sprague-Dawley rats (Hachiya et al. 1985). Male and female mice aged 5-7 weeks and male and female rats aged 5-6 weeks were administered doses up to 5000 mg/kg bw by gavage (with 5-10 animals/sex/species/dose) and observed for 14 days. No deaths or adverse clinical signs were seen in either mice or rats of either sex, and the LD_{50} for male and female mice and rats was >5000 mg/kg bw.

Following OECD guidelines, Noda et al. (1988) administered limit doses of 5000 mg/kg bw of tamarind seed polysaccharide by gavage to 10 male and 10 female ddY mice, 10 male Wistar rats, and 10 male and 10 female Sprague-Dawley rats. All animals were 5 weeks old at the time of the gavage administration. The animals were group-housed in stainless steel mesh cages (10 mice/cage and 5 rats/cage), given free access to feed and water, and observed for 14 days. After sacrifice, they were subjected to gross necropsy. No deaths occurred and no abnormalities were seen in either sex of either mice or rats. The LD_{50} for acute oral toxicity of tamarind seed polysaccharide in male and female rats and mice was >5000 mg/kg bw.

In an unpublished study, Takizawa et al. (1993) assessed the acute oral toxicity of tamarind seed polysaccharide in 9-week-old male and female ddY mice. Tamarind seed

polysaccharide (insoluble in any solvent) was suspended in distilled water at a maximum concentration of 80 mg/ml and administered by gavage to 5 mice at a dose of 25 ml/kg bw, providing 2000 mg polysaccharide/kg bw; control-group animals received equal volumes of distilled water. The animals were observed for 14 days and weighed daily; after sacrifice they were subjected to necropsy to obtain macroscopic findings. There was no mortality, no weight loss or other signs of toxicity were observed in male or female animals, and no abnormalities were detected by necropsy. The LD_{50} for male and female mice was thus >2000 mg/kg bw.

5.2.2. Subacute Oral Toxicity

Based on a dietary assessment, the intended use of tamarind seed polysaccharide as a thickener and stabilizer in foods (e.g., ice cream, sauces and condiments, dressings and mayonnaise, fruit preserves, desserts, beverages, pickles, spreads and fillings, flour products, and soup) is estimated to produce intakes of the polysaccharide as high as 91 mg/kg bw/day among heavy consumers of these foods. A 28-day dietary study was conducted with doses chosen to confirm the safety of this level of exposure (i.e., 91 mg/kg bw/day) to tamarind seed polysaccharide (Heimbach et al. 2013). The study was conducted according to OECD Guideline No. 407 (Repeated Dose 28-Day Oral Toxicity Study in Rodents) and FDA's Redbook 2000 (IV.C.4.a. Subchronic Toxicity Studies with Rodents), and met the requirements of the OECD Principles of GLP and 21 CFR 58: FDA GLP Standards. The stability of the test substance was verified using neat samples taken at the initial, middle, and final diet preparations, which were frozen until analysis of total dietary fiber using the method of AOAC 991.43.

Eighty 7- to 8-week old Crl:Sprague-Dawley CD® IGS rats (40 rats/sex) were used in the study. Body weights ranged from 173-198 g (mean = 185.5±6.6 g) for females and 210-246 g (mean = 228.6±10.4 g) for males at treatment initiation. Rats were assigned to 4 test groups consisting of 10 rats/sex. The animals were individually caged, and diets (2016CM Harlan Teklad Global Rodent Diet®) were fed *ad libitum* for 28 days with tamarind seed polysaccharide admixtures of 0 (control), 40,000, 80,000, and 120,000 ppm to target approximate exposures of 0, 3333, 6667, and 10,000 mg/kg bw/day (Heimbach et al. 2013).

Animals were observed at least twice daily for viability and once daily for cage-side observations of appearance and behavior. Detailed clinical examinations were conducted prior to first treatment and weekly thereafter until study termination. During acclimation and at study termination, mydriatic eye drops were placed into the eyes of the rats which were then examined in subdued light by focal illumination, indirect ophthalmoscopy, and, when indicated, slit-lamp microscopy. Body weight was recorded twice during acclimation, on Day 0 (first day of treatment), weekly thereafter, and just prior to necropsy. Feed consumption was measured weekly to coincide with body weight measurements. A Functional Observational Battery (FOB) was performed on all animals during Week 4. Each rat was evaluated during handling and while in an open field for excitability, autonomic function, gait and sensorimotor coordination (open field and manipulative evaluations), reactivity and sensitivity (elicited behavior) and other abnormal clinical signs including but not limited to convulsions, tremors, unusual or bizarre behavior, emaciation, dehydration and general appearance. In addition, forelimb and hindlimb grip strength and foot splay measurements were taken. During the same time period that the FOB was performed, motor activity (MA) was also monitored in all animals. During Weeks 2 and 4,

blood samples were collected via sublingual bleeding under isoflurane anesthesia from all animals, which were fasted overnight prior to each collection.

Hematology parameters evaluated included erythrocyte count, hematocrit, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, absolute reticulocyte count, total white blood cell and differential leukocyte count, hemoglobin concentration, mean corpuscular volume, red cell distribution width, platelet count, prothrombin time, and partial thromboplastin time. Clinical chemistry parameters included aspartate aminotransferase, alanine aminotransferase, sorbitol dehydrogenase, alkaline phosphatase, total bilirubin, urea nitrogen, blood creatinine, total cholesterol, triacylglycerol, fasting glucose, total serum protein, albumin, globulin, calcium, inorganic phosphorus, sodium, potassium, and chloride. On the day prior to collection of blood samples, rats were fasted for at least 15 hours and then placed in metabolism cages for collection of urine. Urine samples were analyzed for pH, ketone, color, glucose, bilirubin, clarity, specific gravity, blood, volume, protein, urobilinogen, and microscopic urine sediment examination. At the end of the study, all animals were euthanized by exsanguination from the abdominal aorta under isoflurane anesthesia and subjected to a full necropsy including examination of the external surface of the body, all orifices, and the thoracic, abdominal, and cranial cavities and their contents. Vaginal smears were taken from all females to determine estrus stage. Wet organ weights were recorded for adrenals, kidneys, spleen, brain, liver, thymus, epididymides, ovaries, uterus with oviducts, heart, and testes. Epididymides, eyes with optic nerve, testes, prostate, seminal vesicles, ileum with Peyer's patches, salivary glands, adrenals, jejunum, kidneys, all gross lesions, larynx, skeletal muscle, aorta, liver, skin, femur, bone marrow, lungs, skin, spinal cord, lymph nodes, brain, mammary gland, spleen, ovaries, stomach cecum, pancreas, thymus, cervix, sciatic nerve, thyroid/parathyroid, colon, pharynx, trachea duodenum, pituitary, urinary bladder, esophagus, rectum, uterus, vagina, heart, and Harderian gland were excised and examined histopathologically.

All animals survived to study termination. All had normal final ophthalmic examinations. There were no changes in body weight or body weight gain attributable to the administration of tamarind seed polysaccharide. Slight yet statistically significant decreases in body weight gain in mid- and high-dose males during days 0-7 did not persist and were considered incidental due to the palatability of the test substance and therefore of no toxicological significance. The dietary concentrations of 0, 40,000, 80,000, and 120,000 ppm provided mean daily tamarind seed polysaccharide intakes of 0, 3451, 6739, and 10,597 mg/kg bw/day for male rats and 0, 3602, 7190, and 10,691 mg/kg bw/day for female rats, respectively. Mean daily feed consumption and feed efficiency were similar to controls. No statistically significant differences were seen between groups on the FOB or motor activity tests. Incidental differences between groups in the hematology, clinical chemistry, and necropsy measures were not considered toxicologically significant since they were not accompanied by any other corresponding clinical or histopathological changes, were very small, occurred in only one sex, were not dose-dependent, and/or were not significant at study termination. There were no absolute or relative organ weight changes or histopathologic findings attributable to the administration of tamarind seed polysaccharide. Vaginal smears revealed no cyclic changes that might be considered the result of test substance administration.

Since there were no toxicologically significant effects in this study up to the highest dietary concentration tested of 120,000 ppm, the no observed adverse effect level (NOAEL) for

tamarind seed polysaccharide in the diet was considered to be 120,000 ppm, equivalent to 10,597 and 10,691 mg/kg bw/day for male and female rats, respectively.

5.2.3. Subchronic Oral Toxicity

Sano et al. (1996) conducted a 13-week oral toxicity feeding study with 10 $B6C3F_1$ mice/sex/dose consuming Oriental MF basal diet containing 0, 6250, 12,500, 25,000, or 50,000 ppm tamarind seed polysaccharide as a range-finding study for a planned 78-week carcinogenicity study,. The mice were 5 weeks of age and weighed 23.5±1.0 g (males) and 18.6±0.9 g (females) at the start of the feeding period. Housing was not described. Feed and water were freely available. The mice were observed daily for changes in behavior or clinical signs of toxicity, and feed and water consumption and body weight were recorded weekly. The animals were euthanized under ether anesthesia and blood was taken for hematology (red and white blood cell counts, hemoglobin concentration, and hematocrit) and clinical chemistry (total protein, albumin, albumin:globulin ratio, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, bilirubin, and urea nitrogen). Necropsy was performed, and the brain, heart, liver, spleen, kidneys, adrenals, testes, and ovaries were excised and weighed. These organs as well as lymph nodes, bone marrow, thymus, pituitary, thyroids, parathyroids, trachea, lungs, tongue, salivary glands, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, pancreas, gall bladder, urinary bladder, prostate, seminal vesicle, mammary gland, uterus, vagina, femur, sternum, skin and subcutis, eyes, Harder's glands, spinal cord, and grossly visible lesions were prepared for histopathological examination.

No clinical signs or mortality were reported, and no treatment-related adverse effects were noted regarding feed and water consumption or body weight. No hematological effects were seen in either sex in any group; the only significant difference in biochemistry was a slight but statistically significant decrease in total protein levels among males receiving feed with concentrations of 0.625, 2.5, or 5% tamarind seed polysaccharide. This difference, unaccompanied by any differences in other biochemical parameters, was regarded as toxicologically insignificant. No treatment-related changes were observed at necropsy, nor were there significant differences in absolute or relative organ weights or in histopathology. The NOAEL was the highest dietary concentration tested, 5% tamarind seed polysaccharide, equivalent to doses of 8,200 and 10,600 mg/kg bw/day for male and female mice, respectively.

5.2.4. Chronic Oral Toxicity

Iida et al. (1978) conducted a 2-year feeding study to assess the chronic oral toxicity of tamarind seed polysaccharide in male and female Sprague-Dawley rats. Eight-week-old male rats weighing 305 ±5.0 g and females weighing 198 ±2.5 g were assigned to 4 groups with 20 animals/sex/group. The animals were housed individually in metal cages with CE-2 standard diet and water available *ad libitum*; the chow contained 0, 4, 8, or 12% tamarind seed polysaccharide. The animals were observed daily, and feed consumption and body weight were recorded every fourth week. Urine samples were collected during 18-hour confinements in metabolic cages on weeks 6, 19, 31, 43, 57, 67, 85, and 104 for males and 6, 18, 30, 44, 58, 70, 85, and 103 for females. Urinalysis parameters included volume, protein, urea nitrogen, creatinine, sodium, potassium, sodium/potassium ratio, and chlorine. Blood was taken from the tail vein of all surviving animals at week 104 for assessment of hematology (red blood cell counts, white blood

cell counts, differential leukocyte counts, clotting time, hemoglobin, and hematocrit) and from the heart for clinical chemistry (glucose, calcium, phosphorus, urea nitrogen, creatinine, cholesterol, total protein, albumin, bilirubin, alkaline phosphatase, lactic dehydrogenase, aspartate aminotransferase, alanine aminotransferase, sodium, potassium, and chlorine). Gross necropsy was performed on all rats that died or were euthanized during the study or sacrificed at termination. Brain, heart, lungs, liver, kidneys, spleen, pituitary, thyroids, adrenals, testes, uterus, and ovaries were weighed. Paraffin sections were prepared for brain, heart, lungs, liver, kidneys, spleen, pituitary, thyroids, adrenals, testes, uterus, ovaries, aorta, urinary bladder, pancreas, tongue, submaxillary glands, stomach, small and large intestines, parathyroids, prostate, epididymides, skeletal muscle, femoral bone with marrow, peripheral lymph nodes, and any tumor-like masses for histopathological examination.

Based on feed intake and body weight, the actual ingested doses of tamarind seed polysaccharide were:

4% level: 2500 mg/kg bw/day in males, 3100 mg/kg bw/day in females
8% level: 5300 mg/kg bw/day in males, 6700 mg/kg bw/day in females
12% level: 8300 mg/kg bw/day in males, 9400 mg/kg bw/day in females

Feed consumption and body weights did not differ significantly between control and polysaccharide-feeding groups. Among males, mortality over the 2 years was 75% in the control group and 45%, 65%, and 45% in the 4%, 8%, and 12% tamarind seed polysaccharide groups, respectively. Among females, mortality in the control and low, medium, and high dose groups was 45%, 35%, 40%, and 60%, respectively. Despite the apparently large differences in mortality across groups, the authors reported that "the difference between control and [polysaccharide]-feeding groups were not statistically significant." There were no differences between groups in the daily observations, nor in swelling of submaxillary areas, which was seen in about half of the animals of both sexes in each group, beginning at about week 62 and lasting about 1 week. Subcutaneous masses were observed in both sexes, mostly after 80 weeks, in 4, 4, 2, and 2 males and 6, 11, 8, and 6 females of the control, low-dose, mid-dose, and high-dose groups, respectively; the authors reported that these age-related abnormalities are frequently encountered in this strain of rat.

Urinary protein levels increased over the study in both sexes in both control and polysaccharide groups, with no significant differences between groups. No other urinalysis values showed significant changes and none were significantly different between groups. No hematological measures differed significantly between groups. There were no significant differences in biochemistry parameters among male rats; among females, those in the mid-dose group showed small but statistically significant depression in potassium while those in the low- and high-dose groups exhibited slight but statistically significant depression in urea nitrogen and alanine aminotransferase. These differences were not regarded as toxicologically significant.

"Many" (actual numbers not reported) animals of both sexes in the control and polysaccharide-feeding groups showed significantly elevated absolute weights of endocrine organs such as pituitary, thyroids, adrenals, testes, and ovaries, but the authors regarded these changes as unrelated to ingestion of tamarind seed polysaccharide since they occurred as frequently in the control group as in the groups receiving polysaccharide, there was no evidence of dose-dependence, and the increased organ weights were not accompanied by histopathological changes. Many age-associated lesions were observed in control and test animals, most commonly

myocardial degeneration or fibrosis, nephropathy, periarteritis is mesentery and testis, seminiferous atrophy of testis, dilatation of adrenal sinusoids, and pituitary and mammary tumors. With regard to non-neoplastic lesions, myocardial degeneration occurred significantly more frequently among females in the mid-dose group than among controls, but no other type of lesion was seen more frequently in animals of either sex receiving tamarind seed polysaccharide than among control animals. Most lesion types occurred more frequently among controls than among test animals and there was no indication of dose-dependence. The frequencies of neoplastic lesions did not differ significantly across groups.

The authors concluded that, "The occurrence of numerous age-related lesions usually encountered in this strain of rat, including nephropathy, myocardial injury, periarteritis, changes in adrenal cortex, and tumors of pituitary, mammary and adrenal glands were noted in the present study. These lesions appeared with equal frequency in all groups, including control. In conclusion, the data from our 2-year feeding study of GLYLOID [tamarind seed polysaccharide] in rats indicate no toxicity signs in various parameters examined." The NOAEL was the highest dietary concentration tested, 12%, equivalent to 8300 and 9400 mg/kg bw/day in male and female rats, respectively.

5.2.5. Carcinogenicity

Sano et al. (1996) fed 50 male and 50 female 6-week-old B6C3F$_1$mice Oriental MF basal diet containing 0, 1.25, or 5% tamarind seed polysaccharide for 78 weeks. The mice were housed 5/cage and given free access to feed and water. They were observed daily and weighed weekly for the first 14 weeks and biweekly thereafter; feed and water consumption by cage were recorded prior to each weighing. After sacrifice, blood samples were taken (site not reported) and analyzed for red and white blood cell counts, hemoglobin concentration, and hematocrit. Necropsy was performed, and the brain, heart, liver, spleen, kidneys, adrenals, testes, and ovaries were excised and weighed. These organs as well as lymph nodes, bone marrow, thymus, pituitary, thyroids, parathyroids, trachea, lungs, tongue, salivary glands, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, pancreas, gall bladder, urinary bladder, prostate, seminal vesicle, mammary gland, uterus, vagina, femur, sternum, skin and subcutis, eyes, Harder's glands, spinal cord, and grossly visible lesions were prepared for histopathological examination. Full histopathology was performed on mice of both sexes from the control and 5% groups; histopathology on mice in the 1.25% group was performed only on the spleen, lungs, liver, gall bladder, kidneys, and any tissues with abnormal appearance.

No clinical signs related to tamarind seed polysaccharide ingestion were observed during the 78-week feeding period, and no significant differences were seen in either survival or body-weight curves, although weights of treated females were significantly lower than in the control group from week 34 to termination. The authors suggested that, "Although significant body weight gain retardation was found in the 1.25 and 5% groups of females, this seemed to be a beneficial (prevention of obesity) rather than a toxicological adverse effect. This effect was also found with other gums extracted from plants."[1]

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[1] Although the authors did not mention it, the bodyweight gain retardation did not show dose-dependence: the mean final bodyweight in the low-dose group was 11.1% lower than the controls while that of the high-dose group was only 7.2% lower than the controls.

The only difference in hematological measures was a slight but statistically significant decrease in the hemoglobin concentration in male mice in the high-dose group, which was not regarded as toxicologically significant "because of the lack of changes in any other haematological parameters." There were no differences in absolute organ weights, but mean relative weights of the brain, heart, liver, spleen, and kidneys were significantly elevated in female mice receiving tamarind seed polysaccharide. This difference in relative organ weights was attributed to the reduction in body weight among test-group females (as noted, 11.1% and 7.2% in the low-dose and high-dose groups, respectively).

There were no significant differences reported between groups in either sex in the incidence of neoplastic and non-neoplastic lesions or in benign and malignant tumors. All tumors seen were those types considered to be usual in aged $B6C3F_1$ mice. The authors concluded that consumption of tamarind seed polysaccharide, at up to 5% dietary concentration, is "not carcinogenic in either male or female $B6C3F_1$ mice with long-term dietary exposure." Based on the feed intake and body weight of the mice, the 5% dietary concentration produced tamarind seed polysaccharide doses of 6658 and 8575 mg/kg bw/day among males and females, respectively, and the authors regarded 5% dietary concentration as the NOAEL.

5.2.6. Genotoxicity/Mutagenicity

Ishidate et al. (1985) performed chromosomal aberration tests using Chinese hamster lung-derived fibroblast cells on a number of food additives used in Japan, including tamarind seed polysaccharide. Saline solutions containing tamarind seed polysaccharide at a maximum concentration of 2.0 mg/ml were added to cells cultured for 2 days and chromosome specimens were prepared after 24 and 48 hours. One hundred metaphase cells were observed for each concentration and the frequency of cells with chromosomal structural aberrations (chromatid-type and chromosome-type gaps, breaks, exchanges, etc.) and polyploid cells were recorded. No polyploid cells and no cells displaying indications of chromosomal structural aberrations were observed, and the test article was regarded as nonclastogenic under the conditions of the test.

In an unpublished report, Miyabe (1993) described an abbreviated Ames assay of tamarind seed polysaccharide using tester strains *Salmonella typhimurium* TA98 and TA100. Solutions of tamarind seed polysaccharide in distilled water at concentrations of 0, 0.2, 0.5, 1, 2, 5, 10, and 20 mg/plate were tested with and without S9 metabolic activation. No increases were seen in the number of revertant colonies in either tester strain, in either the S9+ or S9- condition, and the test article was regarded as nonmutagenic under the conditions of the test.

In another unpublished report, Kurita (1993) further studied the mutagenicity of tamarind seed polysaccharide by performing a DNA repair test (Rec assay) using *Bacillus subtilis* strains H17 and M45 (respectively, a repair-proficient wild-type strain and a repair-deficient strain). The strains were inoculated into B2 medium and incubated at 37°C, then transferred to Schaeffer's medium and incubated at 37°C to promote spore formation. Spores were harvested, washed, suspended in distilled water, and mixed (with or without S9 solution) with B2-agar medium, which was allowed to solidify. Tamarind seed polysaccharide suspensions in distilled water were prepared at a concentration of 25 mg/ml and diluted to provide dose levels of 15.6, 31.3, 62.5, 125, 250, and 500 µg/disk. A filter-paper disk was impregnated with 20 µl test solution and placed on the agar plate. The plate was incubated at 37°C for 24 hours and the diameter of a

growth inhibitory zone was measured. Kanamycin served as the negative control and mitomycin C as the positive control; the solvent control was distilled water alone.

Tamarind seed polysaccharide did not induce a growth inhibitory zone at any dose level up to the highest dose of 500 μg/disk, regardless of the presence or absence of metabolic activation, in either the wild-type strain (H17) or the repair-deficient strain (M45). The difference in the size of the inhibitory zones between the two strains treated with kanamycin (negative control) was not significant, while mitomycin C (positive control) induced a markedly larger growth inhibitory zone in the repair-deficient strain than in the wild-type strain in both the absence and presence of metabolic activation. In the solvent control plate, no growth inhibitory zone was observed in either strain. Therefore, the test was considered to be valid and tamarind seed polysaccharide was judged negative for bacterial DNA-damage inducibility under the conditions tested.

An Ames assay was conducted under GLP and following OECD Guideline No. 471 (Bacterial Reverse Mutation Test) using *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2 uvrA (Heimbach et al. 2013). Test item concentrations of 10.0, 31.6, 100, 316, 1000, 2500, and 5000 μg/plate were used in both plate incorporation and pre-incubation tests with and without metabolic activation. No biologically relevant increases in revertant colony numbers of any of the five tester strains were observed follow treatment with tamarind seed polysaccharide at any concentration level, either in the presence or absence of metabolic activation in either experiment. The authors of the study concluded that tamarind seed polysaccharide "did not cause gene mutations by base pair changes or frameshifts in the genome of the tester strains used. Therefore, it is considered to be non-mutagenic in this bacterial reverse mutation assay."

5.2.7. Toxicity: Conclusions

The oral toxicity of tamarind seed polysaccharide was evaluated in 5 published and 1 unpublished acute toxicity studies in ddY and $B6C3F_1$ mice and in Wistar and Sprague-Dawley rats, with no mortality at the highest (limit) dose tested, and the LD_{50} was determined to be >5000 mg/kg bw. In a subacute (28-day) feeding study with Sprague-Dawley rats, there were no deaths or signs of toxicity at the tested dietary concentrations of 40,000, 80,000, and 120,000 ppm, equivalent to doses of 3451, 6739, and 10,597 mg/kg bw/day for male rats and 0, 3602, 7191, and 10,691 mg/kg bw/day for female rats. Similarly, the NOAEL was the highest dietary concentration tested—5%, equivalent to 8,200 and 10,600 mg/kg bw/day for males and females, respectively—in a 13-week subchronic oral toxicity study with $B6C3F_1$ mice. In a 2-year feeding study in Sprague-Dawley rats, there was no evidence of toxicity at the highest level tested—12%, equivalent to 8,300 and 9,400 mg/kg bw/day in males and females, respectively. The material was reported to be noncarcinogenic in a 78-week feeding study with $B6C3F_1$ mice at up to 5% dietary concentration (the highest level tested), equivalent to 6,658 mg/kg bw/day for males and 8,575 mg/kg bw/day for females. Tamarind seed polysaccharide was found to be nonmutagenic in both abbreviated and OECD-compliant Ames assays, nonclastogenic in a chromosomal aberration test, and non-DNA-damaging and nonmutagenic in a DNA repair test (Rec assay).

5.3. Other Studies in Animals

Yamatoya et al. (1996) hydrolyzed tamarind seed polysaccharide (xyloglucan) by treating it with endo-1,4-β-glucanase derived from *Trichoderma viride*. Six-week-old male Wistar rats weighing an average of 130 g were randomized to 2 groups of 6 rats/group, both of which received a high-fat diet with 2% corn oil and 15% lard for 35 days. The diet of one group included 5% cellulose while that of the other contained 5% hydrolyzed xyloglucan. All animals had free access to feed and water. At the end of the feeding period, the animals were weighed and, after an overnight fast, blood was collected for analysis of plasma components, the liver was removed and homogenized for analysis of lipids, and adipose tissues were removed from the retroperitoneal and gonadal regions.

Addition of hydrolyzed tamarind seed polysaccharide did not affect feed intake, body weight gain, or liver weight. Adipose tissue weight was significantly reduced in comparison to rats receiving cellulose, as was total blood lipid, total cholesterol, triacylglycerol, and β-lipoprotein as well as hepatic total lipid, cholesterol, triacylglycerol, and phospholipid concentrations. Additionally, the rats receiving hydrolyzed xyloglucan had significantly reduced aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transpeptidase, and lactate dehydrogenase activities. No adverse effects were reported, and the authors concluded that "hydrolyzed xyloglucan acts to ameliorate fatty liver and improve the function of liver under a high-fat diet."

In a similar study, Yamatoya et al. (2000) assigned 4-week-old male Wistar rats weighing an average of 100 g to 3 groups (7 rats/group) to receive high-fat diets (containing 2% corn oil and 15% lard) with the addition of 5% cellulose, intact tamarind seed polysaccharide, or hydrolyzed tamarind seed polysaccharide for 28 days. Feed and water were available *ad libitum*. At the end of the feeding period, the animals were weighed, blood was collected for analysis of plasma components, the liver was removed and homogenized for analysis of lipids, and adipose tissues were removed from the retroperitoneal and gonadal regions.

Feed intake and body weight gain did not differ significantly among the 3 groups, nor did liver weight. Cecum weight was significantly higher in the groups given either intact or hydrolyzed tamarind seed polysaccharide than in the cellulose group, while adipose tissue weight was significantly reduced. In the blood tests, intact xyloglucan—but not hydrolyzed xyloglucan—significantly reduced total cholesterol and β-lipoprotein, while hydrolyzed xyloglucan but not the intact form significantly reduced triacylglycerol. In the liver, total lipid was significantly reduced by both intact and hydrolyzed tamarind seed polysaccharide, but total cholesterol was significantly lower only with intact xyloglucan while triacylglycerol was significantly reduced only by the hydrolyzed article. No adverse effects were reported, and the authors concluded that "the intake of both intact and hydrolyzed xyloglucan improves lipid metabolism in rats."

6. SAFETY ASSESSMENT AND GRAS DETERMINATION

6.1. Introduction

This chapter presents an assessment that demonstrates that tamarind seed polysaccharide is safe, and is also GRAS under the Federal Food, Drug, and Cosmetic Act (FDCA) for direct addition to conventional food as a stabilizer and thickener at a level up to 1.5%. This safety assessment and GRAS determination entail two steps. In step one, the safety of tamarind seed polysaccharide under its intended conditions of use is demonstrated. In the second step, tamarind seed polysaccharide is determined to be GRAS by demonstrating that its safety under its intended conditions of use is generally recognized among qualified scientific experts.

The regulatory framework for establishing whether a substance is GRAS in accordance with Section 201(s) of the Food Drug and Cosmetic Act is set forth under 21 CFR §170.30. This regulation states that general recognition of safety may be based on the view of experts qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food. A GRAS determination may be made either: 1) through scientific procedures under 21 CFR §170.30(b); or 2) through experience based on common use in food, in the case of a substance used in food prior to January 1, 1958, under 21 CFR §170.30(c). This GRAS determination employs scientific procedures established under 21 CFR §170.30(b).

In addition to requiring scientific evidence of safety, a GRAS determination also requires that this scientific evidence of safety be generally known and accepted among qualified scientific experts. This "common knowledge" element of a GRAS determination consists of two components: 1) the data and information relied upon to establish the scientific element of safety must be generally available; and 2) there must be a basis to conclude that there is a consensus among qualified experts about the safety of the substance for its intended use.

The criteria outlined above for a scientific procedures GRAS determination are applied below in an analysis of whether tamarind seed polysaccharide is safe and GRAS for the uses and at the use levels intended.

6.2. Safety of the Intended Use of Tamarind Seed Polysaccharide

A scientific procedures GRAS determination requires first that information about the material establish that the intended use of the material is safe. The FDA has defined "safe" or "safety" for food additives under 21 CFR §170.3(i) as "a reasonable certainty in the minds of competent scientists that the substance is not harmful under its intended conditions of use." This same regulation specifies that three factors must be considered in determining safety. These three factors are:

- The probable consumption of the substance and of any substance formed in or on food because of its use;
- The cumulative effect of the substance in the diet, taking into account any chemically or pharmacologically related substance or substances in such diet; and
- Safety factors which, in the opinion of experts qualified by scientific training and experience to evaluate the safety of food and food ingredients, are generally recognized as appropriate.

An estimated daily intake (EDI) for the material is derived based on the probable human consumption of the material, taking into account any existing sources of consumption of the material. Finally, the EDI for a substance is compared against a level of consumption that has been shown to be reasonably certain to be without harm. As long as the EDI is less than or approximates this level, the substance can be considered safe for its intended use (FDA 1993).

6.2.1. EDI of Tamarind Seed Polysaccharide

As indicated above, 21 CFR §170.3(i) requires that, in evaluating the safety of the proposed use of a new food additive, the probable consumption of the substance and of any substance formed in or on food because of its use be considered. Also to be considered is the cumulative effect of the substance in the diet, taking into account any chemically or pharmacologically related substance or substances in such diet. Thus, because a scientific procedures GRAS determination requires the same quantity and quality of evidence as is required to obtain approval of the substance as a new food additive, a scientific procedures GRAS determination must also consider the probable consumption and cumulative effect of the substance in the diet. The EDI derivation described below provides a conservative estimate of the intake of tamarind seed polysaccharide under its intended conditions of use.

Section 4 described the derivation of the estimated exposure to tamarind seed polysaccharide expected to result from its intended use, which calls for its addition to conventional foods at levels ranging from 0.2% to 1.5%. The estimated mean and 90th percentile daily intakes of tamarind seed polysaccharide are 2.57 and 4.43 g, respectively. Expressed in terms of bodyweight, the estimated mean and 90th percentile daily intakes are 44.9 and 91.0 mg/kg bw, respectively.

6.2.2. Evidence for the Safety of Tamarind Seed Polysaccharide

Since all higher plants contain xyloglucan polysaccharides, xyloglucans are necessarily consumed as part of vegetables and fruits. Additionally, the safety of DSP GOKYO FOOD & CHEMICAL Company's GLYLOID and GLYATE brands of tamarind seed polysaccharide has been evaluated in both *in vitro* and *in vivo* toxicity studies. The oral toxicity of tamarind seed polysaccharide was evaluated in 5 published and 1 unpublished acute toxicity studies in mice and rats, a 28-day study of subacute oral toxicity in rats, a 13-week study of subchronic oral toxicity in mice, a 2-year feeding study in rats, and a 78-week feeding study in mice. No test-article-associated morbidity or mortality was seen in any of these studies and the NOAEL was invariably the highest dietary concentration tested. Tamarind seed polysaccharide was found to be nonmutagenic in abbreviated and OECD-compliant Ames assays, nonclastogenic in a chromosomal aberration test, and non-DNA-damaging and nonmutagenic in a DNA repair test (Rec assay).

6.3. International Approvals of Tamarind Seed Polysaccharide

Tamarind seed polysaccharide is approved as a food additive in Japan and countries in southeast Asia including South Korea and China, and it is also used as food in Taiwan.

6.4. General Recognition of the Safety of the Intended Use of Tamarind Seed Polysaccharide

The proposed use of tamarind seed polysaccharide has been determined to be safe through scientific procedures set forth under 21 CFR §170.30(b). Furthermore, because this safety assessment satisfies the common knowledge requirement of a GRAS determination, this intended use can be considered GRAS.

Determination of the safety and GRAS status of tamarind seed polysaccharide for addition to conventional foods at levels from 0.2% to 1.5% has been made through the deliberations of an Expert Panel consisting of Joseph F. Borzelleca, Ph.D., Walter H. Glinsmann, M.D., and Robert J. Nicolosi, Ph.D. These individuals, who carefully reviewed and evaluated the publicly available information summarized in this document, are qualified by scientific training and experience to evaluate the safety of food and food ingredients.

6.5. Conclusion of the GRAS Expert Panel

We, the undersigned independent Expert Panel members, have individually and collectively critically evaluated the information summarized above and other information deemed appropriate, and unanimously conclude that the intended use of tamarind seed polysaccharide produced in accordance with current Good Manufacturing Practice and meeting appropriate human food-grade specifications, is safe.

We further conclude that the intended use of tamarind seed polysaccharide produced in accordance with current Good Manufacturing Practice and meeting appropriate human food-grade specifications is Generally Recognized as Safe (GRAS) based on scientific procedures.

It is our opinion that other qualified experts would concur with these conclusions.

Signature: (b) (6) Date: 20 May 2013

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